DNA Microarray Analysis of Human Monocytes Early Response Genes upon Infection with *Rickettsia rickettsii*

Chien-Chung Chao

Rickettsiae Diseases Department

Infectious Diseases Directorate

Naval Medical Research Center

Silver Spring, MD

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14. ABSTRACT

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Rickettsiae

- Gram negative coccobacillary bacteria
- Obligate intracellular organisms
- Arthropod-borne
- Cause febrile diseases (mild to life threatening)
- Military importance
  - EpidemicTyphus: Peloponnesian war, Napoleon, WWI, WWII
  - Trench fever: WWI and WWII
  - Scrub typhus: WWII, Vietnam, Camp Fuji
  - Ehrlichiosis: (HME Ft Chaffee; Quantico, Ft Campbell)
  - Spotted Fever: (Ft Chaffee, Ft Bragg, Botswana)

Typhus Group (TG) Rickettsiae

- Epidemic (louse-borne) Typhus: *Rickettsia prowazekii*
- Murine (flea-borne) Typhus: *R. typhi*

Spotted Fever Group (SFG)

- Rocky Mountain spotted fever: *Rickettsia rickettsii*
- Mediterranean spotted fever: *R. conorii*
- African tick bite fever: *R. africae*
- North Asian/Siberian tick typhus: *R. sibirica*

Rocky Mountain Spotted Fever

- Etiologic agent: *R. rickettsii*
- Reported in USA, Canada, Mexico, Costa Rica, Panama, Columbia, and Brazil.
- Most severe SFG rickettsial disease and is the most commonly fatal tick-borne disease in the United States. Case-fatality rates up to 30% were reported in the pre-antibiotic era but rates have remained between 2-10 % since the 1950's.
- Recent Brazilian outbreak, 66% of cases were fatal.
- Seroconversions were observed for military units that trained in Arkansas (38%) and more than 40% of these individuals received medical treatment.

Diagnosis of rickettsial disease

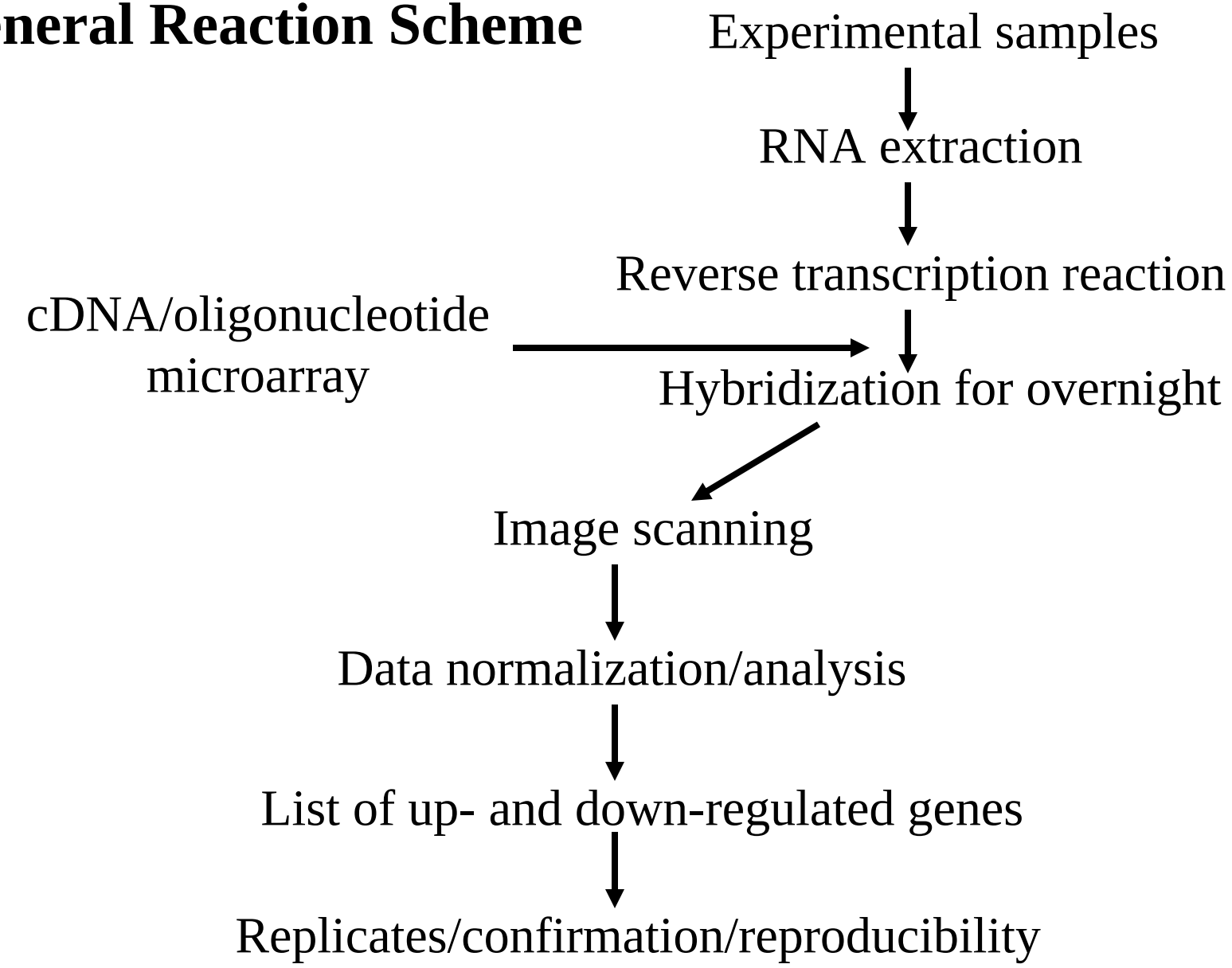
- Generally based on clinical presentation and exposure history of a patient.
- Clinical characterizations are sudden onset of fever, primary eschars, severe headache, myalgia, arthralgia, malaise, and skin rashes.
- Differentiating rickettsial diseases from other acute tropical febrile illnesses can be difficult because of the similarities in signs and symptoms.

Currently available laboratory diagnosis of rickettsial diseases

- serodiagnostic assays such as the indirect immunoperoxidase (IIP) assay and indirect immunofluorescent (IFA) tests (weeks)

- nucleic acid detection by PCR amplification of rickettsial genes (days)

General Reaction Scheme



Hypothesis

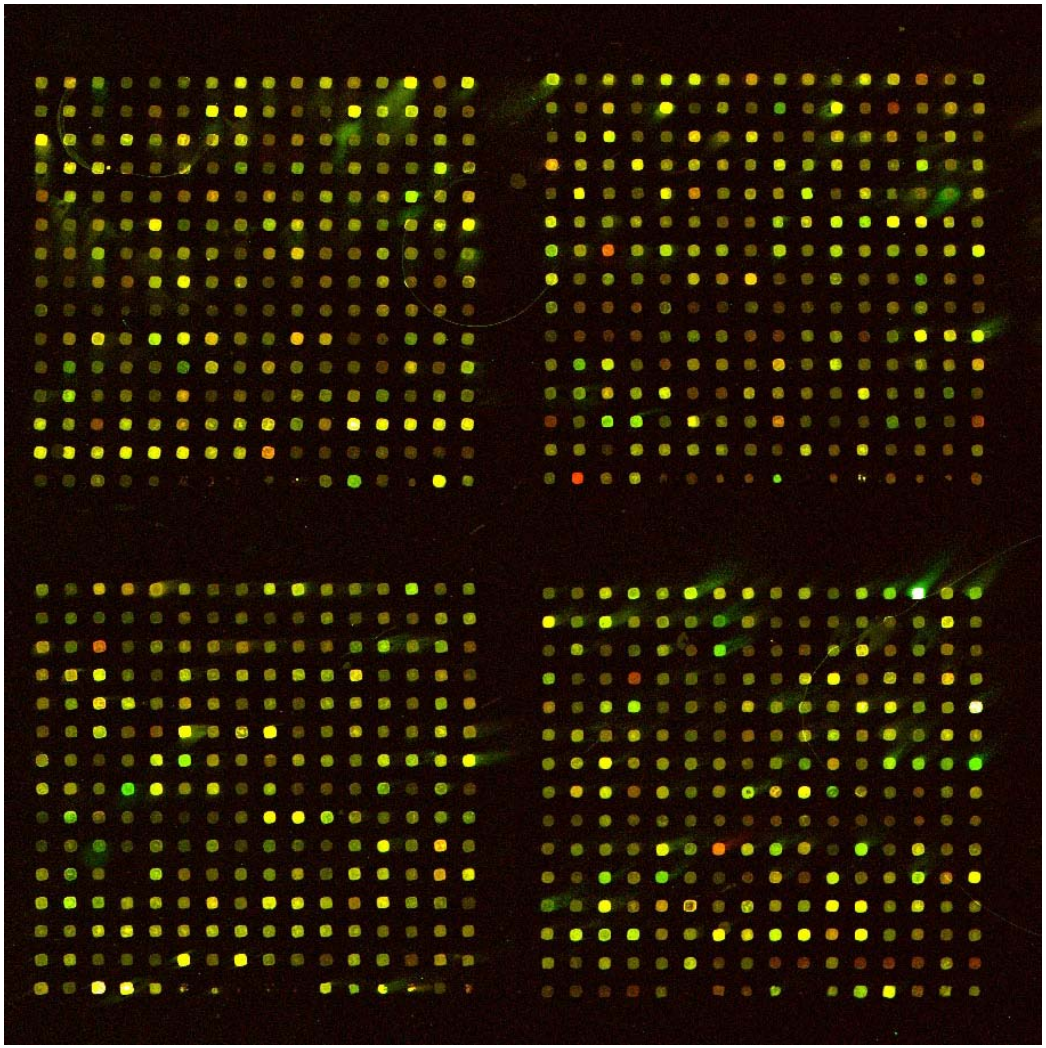
Infection of human monocytes with *R. rickettsii* causes specific mRNA expression pattern. This pattern could be used to distinguish different types of infection and to identify novel genes involved in host response.

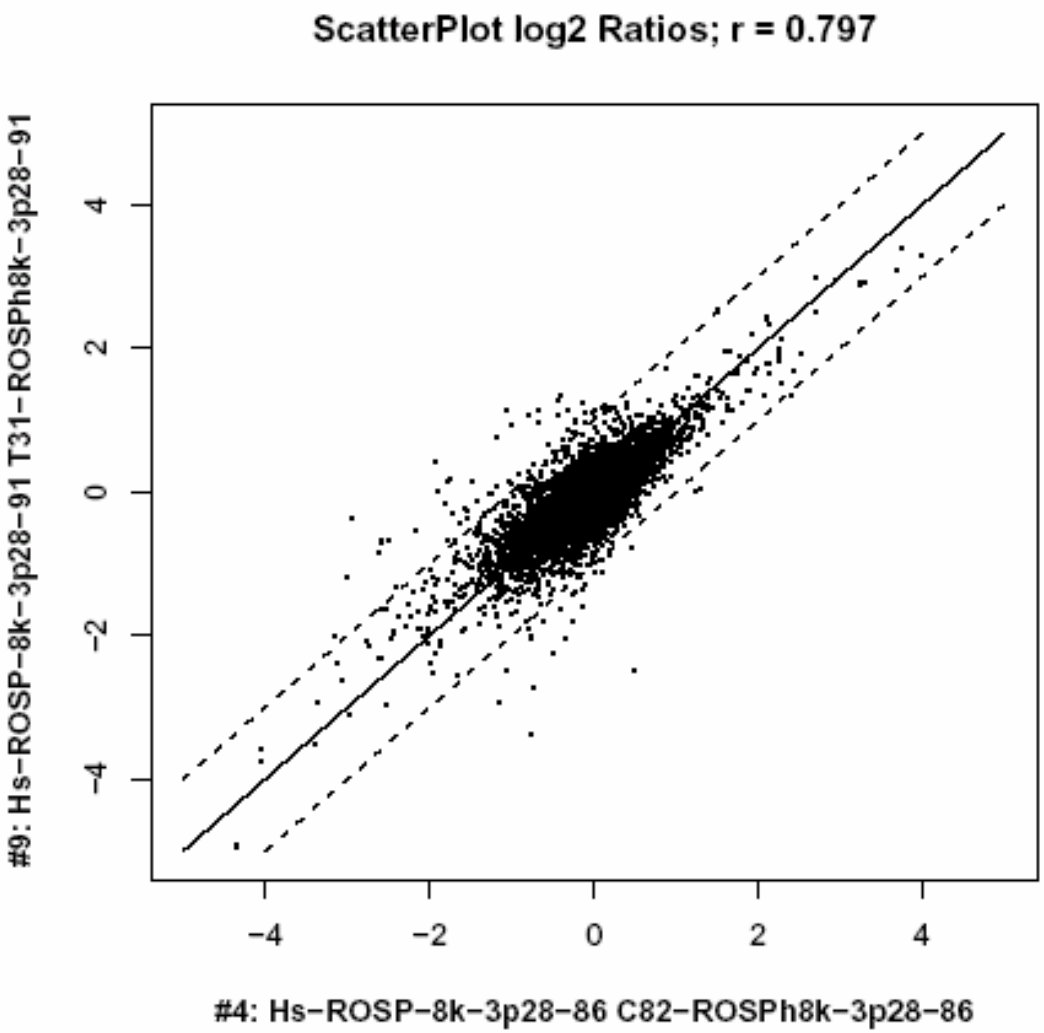
Experimental Design

- Human monocytes (THP-1) were infected with *R. rickettsii* for 45 min in 5% CO_2 incubator at 35^{o}C with gentle rotation.
- Non-associated *R. rickettsii* were removed by centrifugation.
- Infected and control THP-1 cells were left in the incubator for additional 1, 4, 8 and 18 h.
- At indicated time, cells were centrifuged, washed with PBS and RNA was extracted with trizol.
- Trizol extracted RNA was further cleaned with a RNeasy kit.

Experimental Design (continued)

- Reverse transcription reaction was carried out using poly dT and Superscript kit in the presence of Cy3-dUTP (for labeling sample RNA) and Cy5-dUTP (for labeling human reference RNA).
- Labeled RNA was purified and hybridized at 42^{o}C for overnight onto a DNA microarray slide with 7680 human genes.
- The fluorescence images were scanned and visualized using GenePix Pro 4.0 (Axon Lab).
- Data was analyzed using web-based analysis software from NCI (http://nciarray.nci.nih.gov).

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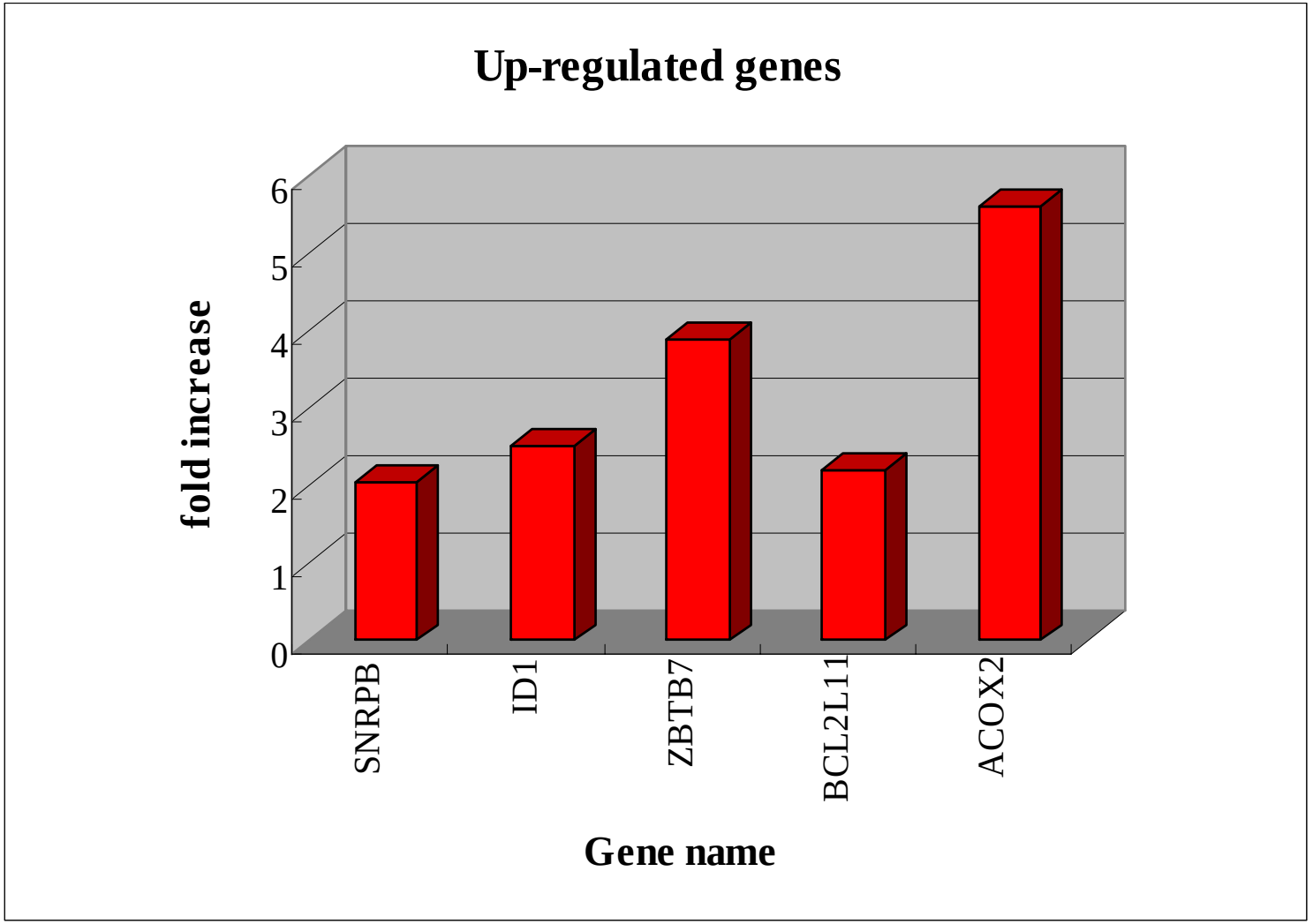
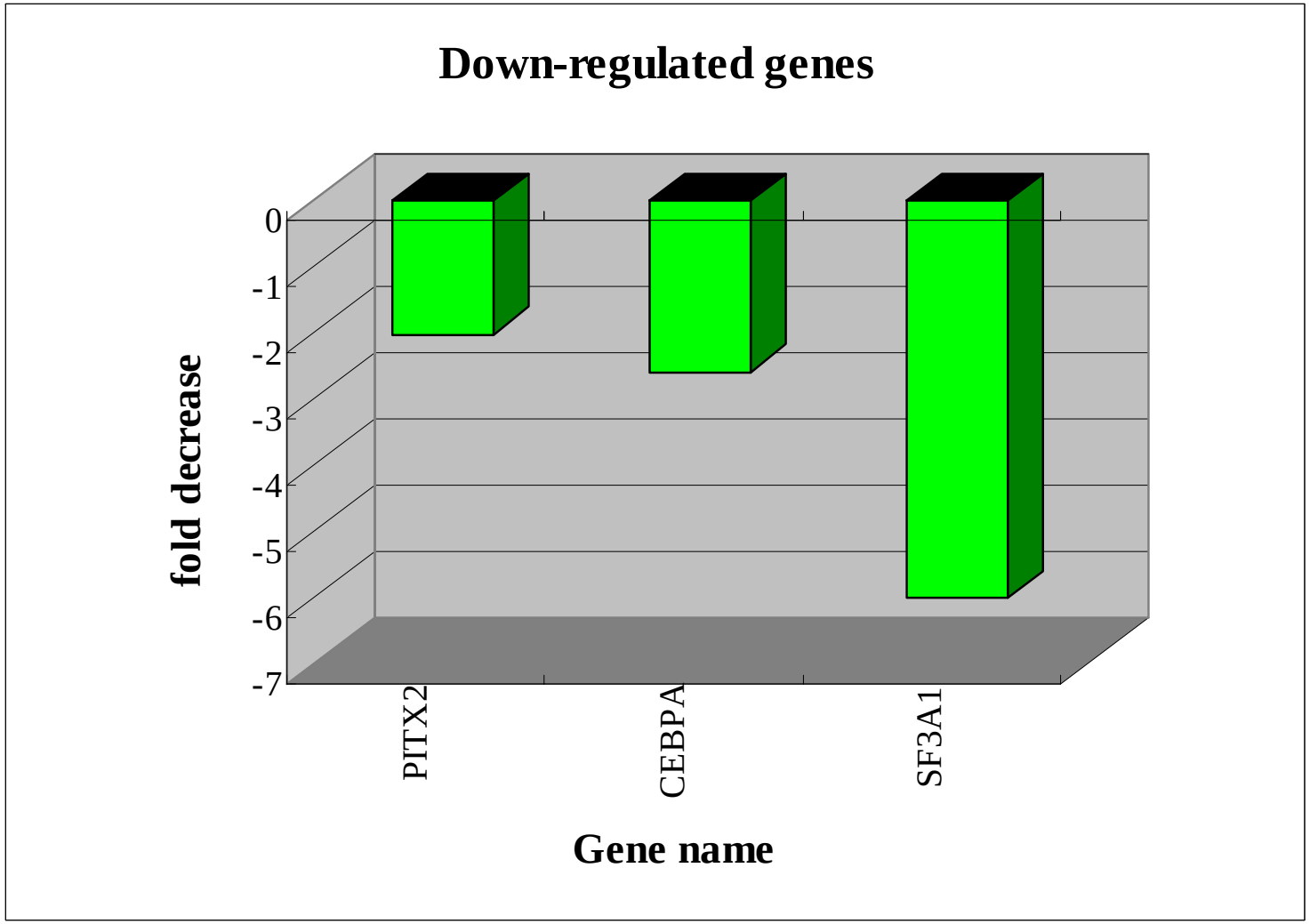
#9: Hs−ROSP−8k−3p28−91 T31−ROSPh8k−3p28−91
#4: Hs−ROSP−8k−3p28−86 C82−ROSPh8k−3p28−86
−4
−2
0
2
4


Up-regulated genes
fold increase
0
1
2
3
4
5
6
SNRPB
ID1
ZBTB7
BCL2L11
ACOX2
Gene name


Down-regulated genes
fold decrease
0
-1
-2
-3
-4
-5
-6
-7
PITX2
CEBPA
SF3A1
Gene name

Comparison of gene lists from various infectious agents

Infectious agents	*Orientia tsutsugamushi*	*R. prowazekii*	*R. rickettsii*	8 others (Nau et. al.)[a]
Orientia tsutsugamushi	20/2[b]	1[c]	0	3
R. prowazekii	---	24/7	0	6
R. rickettsii	---	---	60/25	2
8 others	---	---	---	139/62

a. Data taken from Nau et. al., PNAS, 2002, 99(3), 1503-1508.

b. Presented as the number of up/down regulated genes.

c. Numbers represent genes identified in both infectious agents.

Conclusions

- Infection of human monocytes with *R. rickettsii* resulted in 60 up-regulated and 25 down-regulated genes. These affected genes may be important for the design of diagnostic markers.
- Comparison with microarray results from other infectious agents indicated list of unique genes responsive to *R. rickettsii* infection (Poster by Ge et. al, in Diagnostics session).

Future work

- Confirmation of up- and down-regulated genes.
- Replicates and multiple time points are necessary to identify genes consistently regulated by *R. rickettsii* infection.
- Statistical analysis is needed to better identify genes significantly regulated by *R. rickettsii* infection.

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